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THE PROPORTION OF GRANULAR AND BARRED FORMS OF BACILLUS DIPHTHERIAE IN THROAT CULTURES.*

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IN a preliminary communication[1] in May, 1908, R. G. Perkins reported the occurrence, in the throat cultures submitted to the Bacteriological Laboratory of the City of Cleveland, of a bacillus morphologically very much like certain forms of *B. diphtheriae.* Two forms of such diphtheria-like organisms have been met with, one somewhat larger than the other. Both are characterized by the possession of metachromatic polar granules. Because of the presence of these granules and of the size and shape of the bacilli there is a striking resemblance between these nonpathogenic forms and the types of true diphtheria bacilli labelled "C" and "D" in the exhaustive study upon the morphology of *B. diphtheriae* by Wesbrook, Wilson, and McDaniel.[2] At times there may be a central granule also, or only a single polar granule together with one more centrally situated. Such forms are much like Wesbrook's Type "C." In mixt cultures from the throat, incubated for from 12 to 24 hours, the presence of this organism has been very confusing. Frequently enough, longer incubation and even isolation in pure culture have been necessary definitely to determine whether the organism present in the culture was or was not the nonpathogenic form. With the attention directed to the possibility of the presence of the latter, a proper conclusion is usually reached, even in early cultures, by the fact that the polar granules of the nonpathogenic organism are of such a size as to give the body of the bacillus a cylindrical shape, a characteristic which is just as marked, however, in Wesbrook's Types "C" and "D," and by the absence of so marked a tendency toward parallel arrangement as occurs in *B. diphtheriae.*

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[1] Presented at the Eighth Annual Meeting of the American Association of Pathologists and Bacteriologists, Ann Arbor, Mich.

[2] *Eighteenth Report of the State Board of Health and Vital Statistics of Minnesota,* 1899–1900, p. 613.

That the liability of confusion and the possibility of mistakes in the bacteriological diagnosis of diphtheria might be lessened to a certain degree appeared probable from the following paragraph by F. P. Gorham:[1] "In the routine examinations for the diagnosis of diphtheria in our own laboratory, and Mr. Rickards informs us that the same is true in the Boston laboratory, the granular forms of the diphtheria bacillus have been exceptionally uncommon this fall. Their place is taken by the barred type, which have become now the most common form. Indeed there would hardly be a positive diagnosis at present were we to abide by the former rule to regard the granular types alone positive."

If the proportion of granular forms of the diphtheria bacillus in Cleveland cultures has decreased to such a degree as has occurred in the Providence and Boston cultures, then a diagnosis of diphtheria could be made from the presence of barred forms in the great majority of cases and the possibility of confusing the nonpathogenic organism with *B. diphtheriae* would be lessened in a corresponding degree. To determine this question of the relative frequency of granular and barred forms a record was kept of the prevailing types of the diphtheria bacillus occurring in the cultures submitted to the Bacteriological Laboratory of the City of Cleveland for the period of one year, from April 1, 1908, to March 31, 1909. A period covering one year, rather than one which would give a certain number of positive cultures, was chosen in order to determine whether there might be any relation between season and the frequency of the various types of bacilli found. Since the purpose of the statistics was to gain an idea of the relative frequency of granular and barred forms, solid forms were left out of consideration, and no attempt was made to classify the various types as completely as was done by Wesbrook and his coworkers. Altho no records of all the minor variations which were met with were kept, there were noted a few types of *B. diphtheriae* not described by Wesbrook. Whenever there was a possibility that the organism under consideration might be the nonpathogenic species pure sub-cultures were obtained. Of the morphological varieties not noted by Wesbrook, Wilson, and McDaniel, Type I*a* approaches most nearly their Type "D," differing from the latter chiefly in the more pointed ends and the smaller size of the polar granules. Bacilli of Type I*a* have been grouped together with those of Type I*b* under "pure granular" forms. Other types, which could be classified neither as pure granular nor pure barred forms, contain both granules and bars, are Types II*a*, II*b*, II*c*. These are most like Wesbrook's Types "D," "E," and "F." They differ from the latter in the possession of polar granules and bear the same morphological relation to the latter types that Wesbrook's Type "G" bears to his Type "G^2." Bacilli of Types II*a*, II*b*, and II*c* have been grouped together as "granular-barred" forms, and have been counted under a separate head. If one wished to include them under either of the other two groups, granular or barred, they would more properly belong with the former, since the presence of granules is much more striking than the bars and is more helpful in making a diagnosis. These granular-barred forms may vary in size and in shape, but all are characterized by the possession of polar granules and barred protoplasm. A glance at Table 2 shows that they have been rather frequently encountered. In many of those cultures in which the granular-barred form was the predominating type a few pure granular and pure barred forms were also present. Under the barred types have been included those cultures containing bacilli with barred protoplasm but without granules, size and shape of the organism being left out of consideration, Type III. The bacilli included in

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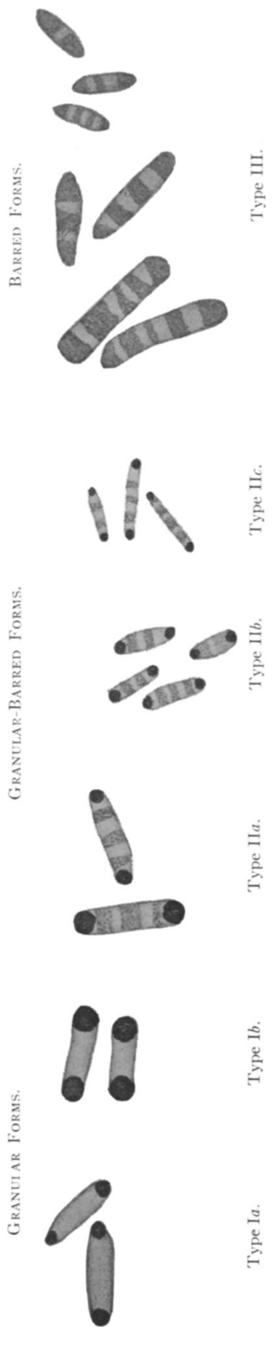
[1] *Amer. Jour. Public Hygiene*, 1907, 17, p. 400.



this group are most like Wesbrook's Types "C^1," "D^1," and "E^1." The so-called involution forms have been included with the granular, barred, or granular-barred forms, depending upon the particular characteristics possessed.

Table 1 gives the total number of cultures examined during the year, together with the total number and percentage of positive results, the number of first cultures, and the number of secondary cultures. The seasonal distribution of positive cultures is the usual one for Cleveland. At no time during the year was diphtheria epidemic.

TABLE 1.

TOTAL CULTURES EXAMINED.

Month	Total Cultures	Total Positive	Percentage of Total Positive	Total 1st Cultures	Positive 1st Cultures	Percentage of Positive 1st Cultures	Total Repeat Cultures	Positive Repeat Cultures	Percentage of Positive Repeat Cultures
1908									
April	34	1	2.9	33	1	3.03	1	0	0.0
May	22	4	18.1	22	4	18.1	0	0	0.0
June	23	7	30.4	17	5	29.4	6	2	33.3
July	24	4	16.6	22	4	18.1	2	0	0.0
August	21	5	23.8	18	4	22.2	3	1	33.3
September	48	13	27.1	41	13	31.7	7	0	0.0
October	89	32	35.9	65	23	35.3	24	9	37.5
November	97	47	48.4	79	41	51.9	18	6	33.3
December	154	45	29.2	109	28	25.6	45	17	37.7
1909									
January	137	27	19.7	112	19	16.9	25	8	32.0
February	103	30	29.1	74	18	24.3	29	12	41.3
March	98	25	25.5	80	20	25.0	18	5	27.7
Totals	850	240	28.0	695	180	25.9	178	60	33.7

Table 2 gives the total number of positive first cultures per month,

TABLE 2.

POSITIVE 1ST CULTURES. PROPORTION OF VARIOUS TYPES.

Month	Total Positive	Granular	Barred	Both Granular and Barred	Granular-barred
1908					
April	1	1	0	0	0
May	4	3	1	0	0
June	5	2	0	3	0
July	4	3	1	0	0
August	4	3	0	1	0
September	13	3	0	3	7
October	23	11	1	7	4
November	41	21	1	9	10
December	28	18	0	1	9
1909					
January	19	11	1	1	6
February	18	15	0	0	3
March	20	12	0	0	8
Totals	180	103	5	25	47
Percentage of various types		57.2	2.7	13.8	26.1

classified according to the type of bacillus which predominated. Pure granular forms occurred with greatest frequency, cultures containing granular-barred forms were next most numerous, pure granular and pure barred types occurred in association in 25 cultures, and pure barred forms alone were met with only in five cases. Such a classification cannot, of course, be very exact. In placing the type encountered in a given culture under any of the four heads the predominating form alone was considered. It is quite certain that in the cultures included under any one group isolated bacilli of other types were also present.

TABLE 3.

POSITIVE REPEAT CULTURES. PROPORTION OF VARIOUS TYPES.

Month	Total Positive	Granular	Barred	Both Granular and Barred	Granular-barred
1908					
April	0	0	0	0	0
May	0	0	0	0	0
June	2	1	0	1	0
July	0	0	0	0	0
August	1	0	0	1	0
September	0	0	0	0	0
October	9	4	0	1	4
November	6	3	0	1	2
December	17	12	0	0	5
1909					
January	8	4	0	0	4
February	12	8	0	1	3
March	5	3	0	1	1
Totals	60	35	0	6	19
Percentage of various types		58.3	0.0	10.0	31.6
Total positive cultures	240	138	5	31	66
Percentage of various types		57.5	2.08	12.9	27.5

In Table 3 are placed the positive secondary cultures. No pure barred cultures were met with among these. The remaining groups bear about the same relation to each other as in the first cultures. In both primary and secondary cultures the proportion of the various types to the total in each group remains approximately uniform.

It will appear at once that the results obtained in the Providence and Boston laboratories do not hold for cultures submitted to the Cleveland laboratory. Whereas, in the Boston and Providence cultures, barred types were so frequent that, according to Gorham, "there would hardly be a positive diagnosis at present were we to abide by the former rule to regard the granular types alone positive," in Cleveland barred forms have recently almost entirely disappeared.

Because of the predominance of granular forms we are still confronted with the possibility of confusing *B. diphtheriae* with the nonpathogenic organism described by Perkins. In connection with the small proportion of barred types in the Cleveland cultures it is interesting to note that the change in the prevailing type seems to be in a direction opposite to that noted in Boston and Providence. There, granular forms seem to have given way to barred types. Here, a comparison of the records of the past year with those of 1902 shows that barred forms have almost disappeared. In 1902, of 236 positive first cultures 94 (36.7 per cent) contained pure barred forms, 29 (11.3 per cent) contained barred and granular types in association, and 133 (51.9 per cent) contained granular forms alone.

What may be the causes underlying the production of a type containing both granules and bars, and what those which lead to changes in the prevailing type in different localities cannot be determined until the biological principles underlying the production of granules and bars in general shall have been better established.

SUMMARY.

Contrary to what has been noted elsewhere, the granular forms of *B. diphtheriae* in throat cultures sent to the Cleveland City Bacteriological Laboratory are increasing in frequency. Barred forms have almost entirely disappeared.

A type not present in earlier years, one not described by Wesbrook, Wilson, and McDaniel, is encountered with considerable frequency. Bacilli of this type contain both granules and bars. They vary in size and shape in cultures from different sources.

Granular types are present in over half of the positive cultures. Next in frequency are the granular-barred forms. In a fair proportion of cases both pure granular and pure barred forms are present in about equal numbers. Pure barred cultures are very infrequent. These proportions hold for secondary as well as for primary cultures.

The predominance of granular forms makes it necessary to bear in mind the occasional occurrence of a nonpathogenic bacillus which, in 12- to 24-hour cultures, is so much like certain of the granular types as to be confusing.